II. REMARKS

Claims 1-20 are pending in the application. Claims 1-20 have been rejected. Claims 1, 5, 6, 10, 11, 15, 17, and 19 have been amended. Claims 4, 9, 12, 13, 14, and 18 have been canceled without prejudice. Applicants respectfully traverse the rejection of claims 1-20.

Applicants are executing a new Declaration of Inventorship, which they believe to be in compliance with 37 C.F.R. § 1.67(a). This Declaration of Inventorship will be forwarded in a separate communication when it is fully executed. Applicants believe this will moot the Examiner's objection.

A. Introduction

The present application is a continuation of United States Application No. 09/374,742. At the close of prosecution in this parent application, a significant amount of prosecution had taken place and progress towards allowance had occurred. Many of the same issues that are addressed in the present office action were addressed and overcome in the parent application. In an effort to provide a complete response to the present rejection, and to address the issues that remained at the close of prosecution in the parent application, Applicants have recreated, but streamlined, the arguments from previous prosecution and have provided copies of the Declarations in support of those arguments. Applicants also address, through a new Declaration and argument, the main issue remaining at the close of prosecution of the parent case, which was the Examiner's belief that there were certain insufficiencies in the first two Declarations by Dr. Klein. These alleged insufficiencies are addressed in the present Declaration. Also, in an effort to facilitate prosecution, and while still maintaining that the claims as originally filed were enabled and fully described, Applicants have amended the claims to read as they did at the close of prosecution of the parent application. Applicants maintain that the arguments, facts, and case law, provided during the earlier prosecution are equally applicable here. Applicants do not

intend to give up a particular argument, even if it is not explicitly recited here, as Applicants have merely tried to summarize the current state for the ease of the Examiner.

B. Rejection Under 35 U.S.C. § 112, ¶ 1

The Office Action dated December 10, 2001, rejected claims 1-11 under 35 U.S.C. § 112, ¶ 1, for allegedly not being enabled for 1) all compounds that produce bisubstrate inhibitors, 2) all acetyltransferases, and 3) for reading on *in vivo* uses.

1. Claims enabled for more than just bromoacetylated bisubstrates

The Examiner appears concerned that the specification allegedly only enables bromoacetylated substrates. First, the claims have been amended to be drawn to bromo-modified substrates and chloro-modified substrates. As discussed below, the application, is fully enabled for any halogenated substrate, and the declaration submitted in the parent application indicates that both bromo and chloro activated substrates work as claimed.

The specification clearly indicates that more than just bromoacetyltryptamines can function as claimed (see, for example, page 3, lines 1-30). It is unequivocal that the specification encompasses the halogens, such as fluorine, chlorine and bromine. Again, as discussed above, the fact that a bromine activated substrate works demonstrates that the acetyltransferases as a class are able to incorporate this type of leaving group into the enzymatic mechanism.

The enclosed copies of the Declarations by Dr. Klein in Exhibits A and B, filed in the parent application, further substantiate the teachings of the specification. In particular, the data presented in Table 1A of the Declaration in Exhibit A and discussed therein clearly show that both bromoacetyltryptamine and chloroacetyltryptamine function intracellularly to inhibit AANAT activity. These data directly address the Examiner's concerns with respect to enablement of more than just bromoacetyltryptamine.

The first Declaration filed by Dr. David Klein under 37 C.F.R. § 1.132 in the parent application, and set forth in Exhibit A, showed that N-chloroacetyltryptamine (CAT) functioned as claimed and stated in the present specification. In the second Declaration submitted by Dr. Klein, set forth in Exhibit B, data showing that additional acetyl acceptor substrates for an acetyltransferase function, as well as showing that additional acetyltransferases can be used to produce the bisubstrate inhibitor are provided.

The Declaration in Exhibit B provides data from studies demonstrating that BAT analogs, i.e. different alkylating derivatives, function to produce a bisubstrate inhibitor in a cell. The Declaration shows that the potency of BAT as an inhibitor of AANAT is a function of the aromatic group by using analogs in which the aromatic groups were different. The effectiveness of the analogs was determined by incubating the compounds with human AANAT. The analogs were dissolved in ethanol and subsequently diluted in sodium phosphate buffer (0.1 M, pH 6.8) containing 2% ethanol to make a stock solution that was 4X, relative to the highest concentration used. This was then diluted as required.

A range of concentrations of the compounds was tested by incubating the compounds (37° C, 15 min) with purified enzyme preparation in the presence of [^{3}H]-acetyl CoA (4 Ci/mol. 0.5 mM) and tryptamine (1 mM) in a total volume of 0.1 ml. Following incubation, the [^{3}H] acetyltryptamine was extracted using chloroform, washed twice using 1 N NaOH (0.2 ml), and radioactivity in 0.4 ml of chloroform was determined. This experiment was done with human AANAT (hAANAT) (results shown in Table 2 and Figure 1 in Exhibit B) and ovine AANAT (data not presented). The identifying numbers (I.D.#) of the analogs and their chemical structures are S 27479-1 (2-Bromo-N-[2-(5-methoxy benzothiophen-3-yl) ethyl] acetamide); S 27535-1 (2-Bromo-N-[2-(5-fluoro benzothiophen-3-yl) ethyl] acetamide); S 27244-1 (2-Bromo-N-[2-(7-hydroxy napht-1-yl) ethyl] acetamide); S 20251-1 (2-Bromo-N-[2-(7-methoxy napht-1-yl) ethyl] acetamide). BAT was tested as a control and

therefore, it was possible to estimate the IC50 values and potency relative to BAT (Table 2, Exhibit B).

The Declaration of Exhibit B states that these results teach that the potency of BAT as an inhibitor of AANAT is a function of the aromatic ring and demonstrate that changing this ring will influence potency. It is also clear from this study that a more potent analog S 27244-1 has the structure of 2-bromo-N-[2-(7-hydroxy napht-1-yl) ethyl] acetamide and teaches that substitution of the indole ring of BAT with the 7-hydroxynaphtyl ring increases potency.

These studies demonstrate that additional acetyl acceptor substrates other than N-bromoacetyltryptamine and N-chloroacetyltryptamine and additional acetyltransferases other than AANAT can successfully be employed in the claimed methods of the present invention to produce a bisubstrate inhibitor by a common mechanism.

C. Other Acetyltransferases can be targeted

The PTO also questions whether other acetyltransferases can be targeted using the claimed methods. The Declaration set forth in Exhibit B shows that the claimed methods work for acetyltransferases other than AANAT as well. The data in the Declaration of Exhibit B demonstrate that bromoacetylkanamycin functions as an alkylating derivative and inhibits kanamycin-6' -N-acetyltransferase. Both bromoacetylkanamycin and kanamycin 6'-N-acetyltransferase are disclosed in Table 1 of the present application. Kanamycin 6'-N-acetyltransferase is a different acetyltransferase than AANAT and bromoacetylkanamycin, which is specific for kanamycin 6'-N-acetyltransferase, is a different alkylating derivative of an acetyl acceptor substrate than N-bromoacetyltryptamine (BAT) and N-chloroacetyltryptamine, both of which are specific for AANAT. Thus, the data shown in the Declaration in Exhibit B clearly indicates that acetyltransferases other than AANAT can be inhibited using the disclosed methods.

It is well known in the art that, in general terms, typical substrates for acetyltransferases consist of two subparts, subpart A and subpart B. Each of these subparts in the normal substrate is recognized by the enzyme; however, typically each enzyme *uniquely* recognizes subpart B. Thus, for example, acetyltransferase E1 accepts A1B1 as a substrate, enzyme E2 accepts A1B2 as a substrate, enzyme E3 accepts A1B3 as a substrate, and so forth. The data provided in the present specification indicate, when generalized to this hypothetical, that E1 accepts A1bromoB1 or A1chloroB1. In other words, the A1 subunit can be modified with a bromine or a chlorine and E1 will still accept the substrate, as long as it is associated with B1. This shows the general mechanism of acceptance of A1bromo or A1chloro at the A1 position.

The specificity of the enzyme:substrate interaction is typically determined by subpart B, in this hypothetical. Thus, the specificity of the E1:substrate interaction is affected by B1 and likewise the E2:substrate interaction is affected by B2. Therefore, one of ordinary skill in the art would understand, without undue experimentation, that once A1bromo or A1chloro was shown to be acceptable, this type of subunit could be associated with any B subunit, B1, B2, or B3, etc., to provide a substrate for any cognate enzyme, E1, E2, or E3, respectively. Thus, on the basis of applicants' discovery that an acetyltransferase could accept a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate, and given that the structure of the cognate substrate for each acetyltransferase was known (see Table 1 of the specification), applicants have demonstrated, and one of ordinary skill in the art would predict, that the methods of the present claims can be practiced with any acetyltransferase having the common mechanism described herein without undue experimentation.

In addition, Applicants point out that in the Advisory Action filed in the parent application, the Examiner had agreed based on the declarations and evidence submitted by the Applicant that the Applicants were entitled to the full breadth of acetyltransferases, because of the common mechanism employed by the bromo and chloro based bisubstrates. The Examiner stated,

> The Declaration convincingly demonstrates enablement for additional acetyl acceptor substrates other than N-bromoacetyltryptamine and N-chloroacetyltryptamine such that the broader scope of the claims reciting a N-bromoacetylated acetyl acceptor substrate or an N-chloroacetylated acetyl acceptor substrate would be considered enabled for claims limited to in vitro applications or methods.

The Examiner then goes on to indicate that the remaining issue was that the previous evidence of in vivo efficacy submitted by the Applicant was unconvincing because the data was collected at a concentration (60 mg/kg) which was allegedly not disclosed in the application. This data requested by the Examiner has been obtained by the Applicant and is discussed below.

D. Claimed method function *in vivo*

The Office Action dated December 10, 2001, rejected claims 15-20 under 35 U.S.C. § 112, ¶ 1, for allegedly not being enabled for a cell comprising 1) all compounds that produce bisubstrate inhibitors, 2) and for reading on in vivo uses of these cells. The declarations in Exhibit A and Exhibit B show that the claimed methods work in vivo.

The Declaration set forth in Exhibit B provides *in vivo* data that demonstrates to one of ordinary skill in the art that the claimed methods are fully enabled to be practiced *in vivo*. The data demonstrate that a method of producing a bisubstrate inhibitor in a cell where the cell is *in vivo*.

The administration of bromoacetyltryptamine to rats results in the inhibition of melatonin production by pinealocytes. The production of melatonin in pinealocytes is directly controlled by the acetyltransferase, AANAT. BAT has been identified as a potential inhibitor of melatonin synthesis, as shown by its inhibitory effects on AANAT in pinealocytes in cell culture. The rats used in these experiments were treated to elevate melatonin production. BAT was administered to these animals and assays for pineal melatonin production in these animals were conducted one

hour after BAT administration. The measurement of pineal melatonin production is an established index of the rate of ongoing melatonin synthesis.

The experiments which provided the data described above were conducted as described herein and as set forth in the Examples in the present application. Specifically, rats (24 days old, ~60 grams) were treated with isoproterenol (1 mg/kg, sc) to elevate melatonin production. Following a 0.5 hour period, animals were treated with BAT (~60 mg/kg) and then were killed one hour later. Pineal glands were removed and homogenized and melatonin was measured by radioimmunoassay, according to assays known in the art and as described in the present application.

Data generated from these experiments demonstrated that treatment with BAT did not alter the general health of the animal, as indicated by visual examination. Furthermore, changes in breathing rate, balance or activity were not detected. However, BAT treatment was shown to result in significantly lower levels of pineal melatonin in these animals (See Table 1, attached hereto as Exhibit B).

These studies show that BAT inhibits melatonin synthesis *in vivo* by producing a bisubstrate inhibitor upon contact with its target enzyme, AANAT, thereby demonstrating that the methods of the present invention can be carried out in a cell *in vivo*.

Clearly, applicants have met the required standard for enablement of *in vivo* use of the claimed methods. In particular, applicants have shown that the claimed methods work in *in vitro* assays, which is indicative of success *in vivo* and they have demonstrated that the claimed methods work *in vivo*, as taught in the specification and as would be reasonably expected by one of skill in the art on the basis of applicants' *in vitro* data.

This data was submitted during the prosecution of the parent application, and in the advisory action mailed on March 5, 2001, the Examiner indicated that this data was not convincing as the amount of BAT used was 60 mg/kg which was allegedly higher than that which was disclosed in the application. Applicants maintain that 60 mg/kg as well as amounts higher were disclosed and were fully enabled, but in an effort to facilitate prosecution, Applicants have provided *in vivo* data in a third declaration, attached herein as Exhibit C, showing *in vivo* success with concentrations much lower than 60 mg/kg.

The Declaration of Dr. Klein provided in Exhibit C, shows the *in vivo* effect of 10 mgs/kg BAT on intracellular AANAT activity in rats. This data shows that 10 mgs/kg BAT suppressed intracellular AANAT activity, as indicated by reduced levels of melatonin in the pineal gland. (See paragraph 4 3rd Klein Declaration, Exhibit C) The results presented in the Declaration in Exhibit C show that isoproterenol (20 mg/kg sc) treatment increased melatonin content of the pineal gland by ~ 10-fold. BAT treatment did not reduce pineal melatonin in control rats, but did reduce this to ~ 25% of the mean value in isoproterenol-treated rats from 42.97 ± 25.63 to 12.43 ± 3.19 pmoles of melatonin per gland (Table 1 Exhibit C). This provides direct evidence that the claimed methods work *in vivo*, reducing the amount of melatonin in the pinocytes in rats.

Thus, for the reasons set forth above, it is applicants' position that the present application is fully enabled for practice of the claimed invention *in vivo* and provides ample guidance for one of ordinary skill in the art in this regard. Applicants have provided extensive guidance throughout the specification to allow one of ordinary skill in the art to administer the compositions and perform the methods of this invention intracellularly, as well as in a subject. Thus, applicants have clearly provided sufficient data and guidance to enable the claims of the present invention.

The Office Action dated December 10, 2001, rejected claims 11-14 under 35 U.S.C. § 112, ¶ 1, for allegedly not being enabled for *in vivo* methods of increasing serotonin. Claims 12-14 drawn to methods of increasing serotonin have been cancelled without prejudice, thus mooting this rejection. While Applicants maintain that the methods of claims 12-14 are fully enabled, these claims were cancelled merely to facilitate prosecution.

For the reasons presented above, applicants believe that the rejections under 35 U.S.C. § 112, first paragraph have been overcome and respectfully request their withdrawal.

E. Rejection Under 35 U.S.C. § 112, ¶ 2

The Office Action dated October 2, 2002, rejected claim 17 under 35 U.S.C. § 112, ¶ 2, for allegedly distinctly pointing out the claimed invention, because claim 17 depended from the "method of claim 15" and claim 15 is a cell not a method. Claim 17 has been amended to refer to a cell rather than a method. Applicants believe this traverses the Examiner's rejection, and respectfully request reconsideration of the claim 17.

F. Rejection Under 35 U.S.C. § 103

The Office Action dated October 2, 2002, rejected claims 1-3, 6-8, and 15-17 under 35 U.S.C. § 103 for allegedly being unpatentable over Kahil et al. J. Am. Chem. Soc., 120:6195-6196 (1998). Claims 1-3, 6-8, and 15-17 under 35 U.S.C. § 103 for allegedly being unpatentable over Kahilil et al. J. Biol. Chem., 273(46):30321 (1998).

The teachings of Khalil et al. (June, 1998) fail to render the claimed methods and cells obvious. Khalil et al. (June, 1998) teaches a "bisubstrate analog" called compound 1 which consists of serotonin covalently linked to acetyl-CoA. Although it is acknowledged that Khalil et al. (June, 1998) demonstrated that compound 1 was an inhibitor of AANAT, it is also important to note that the assay for inhibition used in the studies described in Khalil et al. (June,

important to note that the assay for inhibition used in the studies described in Khalil et al. (June, 1998) was not and indeed could not have been a cellular assay because the authors of Khalil et al. (June, 1998) specifically state that compound 1 cannot penetrate a cell membrane (page 6196, second column, first full paragraph). Thus, this reference not only fails to teach a method of producing a bisubstrate inhibitor in a cell or a method of inhibiting the activity of an acetyltransferase in a cell, Khalil et al. (June, 1998) also contains no mention whatsoever of a cell comprising a bisubstrate inhibitor and provides no expectation that one of skill in the art could produce such a cell. These authors actually teach away from the claimed invention by describing the inability to get compound 1 into a cell as a major drawback to the use of compound 1 as an AANAT inhibitor *in vivo*: "Given the general inability of acetyl-CoA and other CoA analogues to penetrate cell plasma membranes,[14] it is likely that compound 1 in its current form would be unable to block melatonin production *in vivo*. Of concern in future work will be developing analogues of 1 that can enter cells and inhibit AANAT in living organisms." (page 6196, second column, first full paragraph).

Thus, Khalil et al. (June, 1998) does nothing more than suggest the utility of employing acetyltransferase inhibitors in therapeutic roles in mood and sleep disorders and describes a "bisubstrate analog" which inhibits AANAT but cannot be put into a cell. Although Khalil et al. (June, 1998) identifies the problem of the inability of compound 1 to be taken into cells and suggests that future work focus on developing analogues of compound 1 that can enter cells, there is no teaching or suggestion which would guide an artisan to solve the problem presented in this reference by using an alkylating derivative of an acetyl acceptor substrate without acetyl CoA as an acetyltransferase inhibitor. Furthermore, Khalil et al. (June, 1998) provides no teaching or suggestion whatsoever to introduce into a cell an exogenous nucleic acid encoding an acetyltransferase.

In contrast, the present claimed methods describe the surprising discovery of a method of inhibiting acetyltransferases within a cell by introducing an alkylating derivative of an acetyl

acceptor substrate into a cell containing the acetyltransferase. The alkylating derivative of the claims is readily distinguished from compound 1 of Khalil et al. (June , 1998) in that the acetyl CoA is not covalently linked to the substrate outside the cell, but instead is provided as an endogenous source by the cell and thus, entry into the cell is not inhibited. Thus, it is applicants' own discovery that provides a solution to the problems set forth in Khalil et al. (June, 1998), which is in no way taught or suggested in the cited art.

For the reasons stated above, applicants believe that the present rejection is overcome and respectfully request its withdrawal and allowance of the pending claims to issue.

Claims 1-3, 6-8 and 15-17 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Khalil et al. (November, 1998).

The teachings of Khalil et al. (November, 1998) fail to render the claimed methods and cells obvious. Khalil et al. (November, 1998) teaches a study of the activity of AANAT in the presence of various substrate analogs and in particular describes α-trifluoromethyltryptamine as one of the substrate analogs analyzed. This fluorine-substituted analog of tryptamine is noted to have modest inhibitory action (page 30325, second column, second full paragraph) as demonstrated in a noncellular inhibition assay of AANAT activity (page 30322, second column, last paragraph). However, Khalil et al. (November, 1998) provides no teaching or suggestion to produce a bisubstrate inhibitor in a cell or inhibit the activity of an acetyltransferase in a cell by introducing into the cell an alkylating derivative of an acetyl acceptor substrate for an acetyltransferase in the cell and using Acetyl CoA within the cell to form the bisubstrate inhibitor.

Specifically, the α-trifluoromethyltryptamine disclosed in Khalil et al. (November, 1998) can be clearly distinguished from the alkylating derivative of an acetyl acceptor substrate of the claimed subject matter on the basis that α-trifluromethyltryptamine is well known in the art to be

relatively stable and unreactive. This compound does not contain an acylating group (trifluoromethly is not an acylating group) and therefore, it will not attack chemical bonds. Thus, one skilled in the art would recognize that α-trifluoromethyltryptamine would be unreactive towards CoA-SH. The artisan would also readily recognize that trifluoromethyltryptamine is not an acetyl acceptor in the context of the enzyme, as demonstrated by direct experimentation in the Khalil et al. reference. Neither is it an alkylating derivative of an acetyl acceptor substrate as claimed in the present invention, because it does not contain a reactive group, such as the bromoacetyl group which confers alkylating character.

Furthermore, applicants note that Khalil et al. (November, 1998) provides no teaching or suggestion whatsoever to introduce into a cell an exogenous nucleic acid encoding an acetyltransferase.

Applicants also point out that, even if the disclosure of Khalil et al. (November, 1998) can be even remotely interpreted to suggest trying to use an alkylating derivative of an acetyl acceptor substrate (which α-trifluoromethyltryptamine is not, as pointed out above), for producing a bisubstrate inhibitor in a cell or for inhibiting the activity of an acetyltransferase in a cell, as claimed in the present invention, there is no teaching or suggestion in Khalil et al. (November, 1998) which would provide one of skill in the art with a reasonable expectation of success in such an endeavor, because this reference does not disclose any teaching or suggestion of producing a bisubstrate inhibitor within a cell by employing an exogenous alkylating derivative of an acetyl acceptor substrate and endogenous acetyl CoA.

It is well established in the case law that a proper analysis under 35 U.S.C. § 103 requires consideration of whether the prior art would have revealed to the person of ordinary skill in the art at the time of the invention that he would have a reasonable expectation of success in carrying out the claimed method. See In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438, 1442 (Fed. Cir.

1991). ("Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed [invention]; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success."); In re Dow Chemical Co., 837 F.2d 469, 5 U.S.P.Q. 2d 1529, 1531 (Fed. Cir. 1988) ("The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure.").

Thus, applicants contend that the teachings of Khalil et al. (June, 1998) and/or the teachings of Khalil et al. (November, 1998), either alone or in combination, fail to render the claimed invention obvious and therefore, applicants respectfully request withdrawal of this rejection and allowance of the pending claims to issue.

Applicants also respectfully point out that the rejections set forth above state that the claimed invention would have been *prima facie* obvious to one skilled in the art, based only on the teachings of the art cited in the Office Action. Applicants also wish to point out that the Examiner has already stated, as also set forth above, that the claimed invention is sufficiently unpredictable to be lacking enablement, absent a specific showing of data demonstrating the embodiments encompassed in the claimed invention. Thus, the Examiner is simultaneously stating that the claimed invention is too unpredictable to be expected to work as claimed absent a full showing of data and that the claimed invention is predictably obvious, on the basis that the prior art teachings would have led an artisan to expect to successfully carry out the invention as claimed, based on nothing more than prior art disclosures.

reasonable expectation of success in carrying out the claimed invention and that the Examiner herself has argued that the invention as claimed is unpredictable and thus not enabled absent specific data encompassing the full scope of the claims, thereby establishing the absence of a reasonable expectation of success. Thus, in essence, the Examiner has stated that the claimed invention is not obvious. Therefore, applicants respectfully request the withdrawal of this rejection and allowance of the pending claims to issue.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of the application to issue.

No additional fees are believed due, however, the Commissioner is hereby authorized to change any additional fees that may be required or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date shown below.

Gwendolyn D. Spratt

1-2-03
Date

Appendix 1

Marked up specification and claims

--[0068] Pineal cell preparation and treatment: Pinealocytes were prepared from rat pineal glands by trypsinization as previously described (10). The cells were suspended in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum and maintained (37.degree. C.) for 18 h in a gas mixture of 95% air and 5% CO.sub.2. During this 18 hour period, some cells were treated, as indicated in Table 2 experiment 2, in aliquots of 50,000 cells/300 .mu.l and washed prior to addition of fresh medium and further treatment. In some cases, as in Table 3, experiment 1, cells were not aliquoted until after 18 hours of control incubation and in these cases, aliquots of cells (50,000 cells/300 .mu.l) were prepared and treated with drugs. Drugs were prepared in 100.times.concentrated solutions in water or dimethyl sulfoxide. The duration of the drug treatment was 5 h.—

Please replace Table 3 found beginning at line 1 of page 30 with the following Table 3.

TABLE 3. Effect of *N*-bromoacetyltryptamine on melatonin production by norepinephrine-treated pinealocytes and effects on stimulation of AANAT activity. Cells were prepared and treated as described herein. Experiment 1 shows that 0.1 or 1.0 μM *N*-bromoacetyltryptamine treatment inhibits melatonin production during a 5 hour test period. Experiment 2 shows that, after an 18 hour treatment period with 0.5 μM *N*-bromoacetyltryptamine(BAT) and subsequent wash out to remove the drug, pinealocytes are still able to respond to norepinephrine (NE) with an increase in AANAT activity, indicating that they have not been killed by prior treatment.

Experiment 1.

Treatment of pinealocytes in culture (18-24 hours)	**Melatonin production (pmol/100,000 cells, 18-24 hours)**
Control	Not detectable
Norepinephrine (10 μM)	12.43 ± 2.00
N-Bromoacetyltryptamine (1 μM)	0.65 ± 0.05
Norepinephrine (10 μM) +*N*-Bromoacetyltryptamine (1 μM)	0.95 ± 0.25
Norepinephrine (10 μM) +*N*-Bromoacetyltryptamine (0.1 μM)	4.55 ± 0.04

Experiment 2.

Treatment I (0-18 hr)	**Treatment II (18-23 hr)**	**Melatonin (18-23 hr) (pmole/10^5 cells)**	**AANAT (23 hr) (μmol/h/10^5 [5] cells)**
DMSO	Control	1.12 ± 0.31	ND
DMSO	NE 10 μM	10.44 ± 1.07	0.89 ± 0.10
DMSO	NE 10 μM + BAT (0.5μM)	1.88 ± 0.82	0.48 ± 0.05
BAT (0.5 μM)	Control	1.78 ± 0.14	ND
BAT (0.5 μM)	NE (10 μM)	12.22 ± 2.90	0.86 ± 0.06

In the claims

1. (amended) A method of producing a bisubstrate inhibitor in a cell, comprising introducing into the cell [an alkylating derivative of an] a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate for an acetyltransferase present in the cell.

5. (amended) The method of claim 1, wherein the acetyltransferase is arylalkylamine N–acetyltransferase (AANAT) and [the alkylating derivative of] the acetyl acceptor substrate is selected from the group consisting of N-bromoacetyltryptamine, N–bromoacetylserotonin, –bromoacetylphenylethylamine, N–bromo-acetyl-methoxytryptamine, N-bromoacetyltyramine, N-chloroacetyltryptamine, N–chloroacetylserotonin, N-chloroacetylphenylethylamine, N–chloro-acetyl-methoxytryptamine, and N-chloroacetyltyramine[, N-fluoroacetyltryptamine, N–fluoroacetylserotonin, N-fluoroacetylphenylethylamine, N–fluoro-acetyl-methoxytryptamine and N-fluoroacetyltyramine].

6. (amended) A method of inhibiting the activity of an acetyltransferase in a cell, comprising introducing into the cell [an alkylating derivative of an] a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate for an acetyltransferase present in the cell under conditions whereby a bisubstrate inhibitor will be produced, thereby inhibiting the activity of the acetyltransferase in the cell.

10. (amended) The method of claim 6, wherein the acetyltransferase is arylalkylamine N-acetyltransferase (AANAT) and the alkylating derivative of the acetyl acceptor substrate is selected from the group consisting of N-bromoacetyltryptamine, N-bromoacetylserotonin, –bromoacetylphenylethylamine, N-bromo-acetyl-methoxytryptamine, N-bromoacetyltyramine, N-

chloroacetyltryptamine, N-chloroacetylserotonin, N-chloroacetylphenylethylamine, N-chloro-acetyl-methoxytryptamine, and N-chloroacetyltyramine[, N-fluoroacetyltryptamine, – fluoroacetylserotonin, N-fluoroacetylphenylethylamine, N-fluoro-acetyl-methoxytryptamine and N-fluoroacetyltyramine].

11. (amended) A method of inhibiting melatonin production in a cell which produces melatonin, comprising introducing into the cell [an alkylating derivative of the] a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate of AANAT which is selected from the group consisting of N-bromoacetyltryptamine, – bromoacetylserotonin, N-bromoacetylphenylethylamine, N-bromo-acetyl-methoxytryptamine, N-bromoacetyltyramine, N-chloroacetyltryptamine, N-chloroacetylserotonin, – chloroacetylphenylethylamine, N-chloro-acetyl-methoxytryptamine, and N-chloroacetyltyramine[, N-fluoroacetyltryptamine, N-fluoroacetylserotonin, – fluoroacetylphenylethylamine, N-fluoro-acetyl-methoxytryptamine and N-fluoroacetyltyramine].

15. (amended) A cell comprising a bisubstrate inhibitor, wherein the bisubstrate inhibitor comprises [an alkylating derivative of an] a N-bromoacetylated acetyl acceptor substrate or a N-chloroacetylated acetyl acceptor substrate for an acetyltransferase present in the cell and CoA.

17. (amended) The [method] cell of claim 15, wherein the acetyltransferase is produced in the cell from an exogenous nucleic acid encoding the acetyltransferase.

19. (amended) The cell of claim 15, wherein the acetyltransferase is arylalkylamine – acetyltransferase (AANAT) and the [alkylating derivative of the] acetyl acceptor substrate is selected from the group consisting of N-bromoacetyltryptamine, N-bromoacetylserotonin, – bromoacetylphenylethylamine, N-bromo-acetyl-methoxytryptamine, N-bromoacetyltyramine, N-

chloroacetyltryptamine, N-chloroacetylserotonin, N-chloroacetylphenylethylamine, N-chloro-acetyl-methoxytryptamine, and N-chloroacetyltyramine[, N-fluoroacetyltryptamine, –fluoroacetylserotonin, N-fluoroacetylphenylethylamine, N-fluoro-acetyl-methoxytryptamine and N-fluoroacetyltyramine].